

Hepatotoxic and Nephrotoxic Effects of *Moringa Oleifera* Leaves Extract in Adult Wistar Rats

Ekundina V.O^{1*} Ebeye O.A² Oladele A.A¹ G. O. OSHAM²

1.Department of Medical Laboratory Science, Afe-Babalola University, Ado-Ekiti, Ekiti State

2.Department of Human Anatomy and Cell Biology Delta State University, Abraka.

Corresponding Author address:Kemvic30@gmail.com

Abstract

The tree, *M. oleifera* (*Moringaceae*), is cultivated widely around the world and used for various purposes one of which is as a feed supplement to livestock. The aim of this project is to evaluate the effect of *Moringa oleifera* on the liver and kidney. Twenty (20) adult wistar rats were used and were divided into four groups. Group I which is control received only feed and water. Groups 2, 3 and 4 were administered with 400mg/kg, 600mg/kg and 800mg/kg *Moringa oleifera* respectively. The rats were sacrificed by chloroform anaesthesia. An abdominal dissection was carried out to expose the major blood vessels and internal organs and blood was collected with the aid of a needle and syringe by cardiac puncture. Biochemical analysis; Alkaline phosphate and creatinine; haematological analysis and tissue processing was carried out on liver and kidney. The result showed there was a significant increase in haematological indices, significant decrease in serum creatinine level, no significant effect on alkaline phosphate. This study suggests that *Moringa oleifera* is useful in maintaining the haematological indices and has no untoward effect on the kidney and liver.

Keywords: Alkaline Phosphate, Creatinine, Haematology, *M. oleifera* (*Moringaceae*) and histopathology.

INTRODUCTION

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes (Agaie, 2004). Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness (Newman, 2000). One such plant, *Moringa oleifera* Lam., (Family: *Moringaceae*) is a multipurpose tree, used as vegetable, spice, a source of cooking and cosmetic oil and as a medicinal plant (Fahey, 2005; Fuglie, 1999). It is known as Drumstick in English, Zogallagandi in Hausa, Zogali in Nupe, Okwe oyibo in Igbo, Ewe igbale in Yoruba (Mann, 2003; Keay *et al.*, 1964). All parts of the *Moringa* tree are edible and have long been consumed by humans. The rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective. However, over the past decade, several news-catching episodes in developed communities indicated adverse effects, sometimes life-threatening, allegedly arising as a consequence to taking herbal products or traditional medicines from various ethnic groups. Despite the popular use of *Moringa oleifera* for treating various disorders, there is limited or no scientific data available regarding safety aspects of this remedy, nor are there any documented toxicological studies that can be used to ascertain the safety index of its herbal preparation (Awolede, 2010). Toxicity testing on animals is commonly used to assess potential health risk in humans caused by intrinsic adverse effects of chemical compounds/plants extracts (Afolayan, 2009). Therefore, this present study aimed to carry out extensive toxicological evaluation of the ethanolic leaf extract of *Moringa oleifera* on Wistar albino rats.

Moringa Oleifera

Plant Description

Moringa oleifera Lam (syn. *M. pterygosperma* Gaertn.) is one of the best known and most widely distributed and naturalized species of a monogeneric family *Moringaceae* (Nadkarni, 1976; Ramachandran *et al.*, 1980). The tree ranges in height from 5 to 10 m (Morton, 1991). It is found wild and cultivated throughout the plains, especially in hedges and in house yards, thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams (The Wealth of India, 1962; Qaiser, 1973). It can grow well in the humid tropics or hot dry lands, can survive destitute soils, and is little affected by drought (Morton, 1991).

It tolerates a wide range of rainfall with minimum annual rainfall requirements estimated at 250 mm and maximum at over 3000 mm and a pH of 5.0–9.0 (Palada, 2003). In the Philippines, it is known as ‘mother’s best friend’ because of its utilization to increase woman’s milk production and is sometimes prescribed for anemia (Estrella *et al.*, 2000; Siddhuraju, 2003).

Preparation of Plant Materials

The plants collected were spread on a clean surface and allowed to air dry under room temperature for two weeks. The dried *Moringa* leaves were grinded to powder form using electric grinding machine and weighed

(100g).

Ethanol Extraction of Plant

The 100g powdered *Moringa oleifera* was soaked in methanol of 750ml for 72hrs. The extract was obtained using crude maceration method of extraction. The solvent was extracted under heat using a multi-heating mantle and pressure and a paste like extract was obtained and oven dried to complete solid and grinded to powder. (Ajibade, et al, 2012).

Statistical Analysis

The results were expressed as Mean \pm SEM (standard error of the mean) and statistical significance of the treatment effect was analyzed using the student's t-test statistics (Turkey HSD t-test), one way analyses of variance (ANOVA), followed by post Hoc Turkey's test for multiple comparison, using software social science (SPSS) version 20 windows software and significance at p values < 0.05 while P value > 0.05 were considered to be statistically non-significant.

RESULTS

Weight

Table 1: Effect of Ethanolic Extract of *Moringa oleifera* on Weight of Wistar Rats

Group	Treatment Design	WT Initial	WT Ist Wk	WT 2 nd Wk	WT 3 rd Wk	Kidney WT	Liver WT
1.	Control	123.00 \pm 11.23	131.25 \pm 17.29	138.25 \pm 17.29	145.00 \pm 14.72	0.97 \pm 0.29	4.68 \pm 0.05
2.	400mg/kg	168.25 \pm 33.23 ^a	204.75 \pm 56.22 ^a	201.75 \pm 38.41 ^a	195.50 \pm 36.53 ^a	0.89 \pm 0.26	5.58 \pm 1.37 ^b
3.	600mg/kg	156.00 \pm 16.57 ^a	173.88 \pm 35.30 ^a	187.00 \pm 41.87 ^a	193.75 \pm 34.73 ^a	0.93 ^b \pm 0.32 ^b	5.03 \pm 0.25 ^b
4.	800mg/kg	178.75 \pm 14.93 ^a	178.25 \pm 14.68 ^a	190.00 \pm 23.81 ^a	196.25 \pm 30.92 ^a	0.71 \pm 0.20 ^b	6.90 \pm 1.00 ^a

The mean difference is significant at P=0.05 level, n=5

WT represent weight, WK represent week

a=values are significantly different from control at P \leq 0.05

b=values are not significantly differently from control at P \leq 0.05

Haematological Parameters

Table 2: Effect of Ethanolic Extract of *Moringa oleifera* on Haematological Parameter of Wistar rats

Group	Dose	Haematological Parameters			
		PCV%	HB %	WBC _{TOTAL} Cells/mm ³	RBCT _{TOTAL} Cells/mm ³
1.Control	-	42.25 \pm 6.55	15.06 \pm 2.20	3450 \pm 46.58	7.54 \pm 0.54
2.	400mg/kg	46.75 \pm 5.56 ^b	15.57 \pm 0.06 ^b	3575 \pm 36.40 ^b	7.79 \pm 0.93 ^b
3.	600mg/kg	50.25 \pm 2.87 ^b	17.00 \pm 1.05 ^b	3550 \pm 58.23 ^b	8.50 \pm 0.52 ^b
4.	800mg/kg	47.63 \pm 4.73 ^b	15.93 \pm 1.14 ^b	3875 \pm 94.65 ^b	8.04 \pm 0.57 ^b

The mean difference is significant at P=0.05 level,

Values are means \pm standard deviation (n=5 at each group)

b=values are not significantly differently from control at P \leq 0.05

Biochemical Analysis

Table 3: Effect of Ethanolic Extract of *Moringa oleifera* on Biochemical Parameter of Wistar rats

Group	Dose	Biochemical Parameters	
		Creatinine (mg/dl)	Alkaline Phosphatase (mg/dl)
1. Control		0.88 ± 0.10	19.00 ± 3.16
2.	400mg/kg	0.75 ± 0.06 ^a	21.00 ± 4.08 ^b
3.	600mg/kg	0.75 ± 0.06 ^a	20.50 ± 2.38 ^b
4.	800mg/kg	0.72 ± 0.09 ^a	19.25 ± 2.63 ^b

The mean difference is significant at P=0.05 level,

Values are means ± standard deviation (n=5 at each group)

a= values are significantly different from control at P ≤ 0.05

b= values are not significantly different from control at P ≤ 0.05

Histology of the Kidney

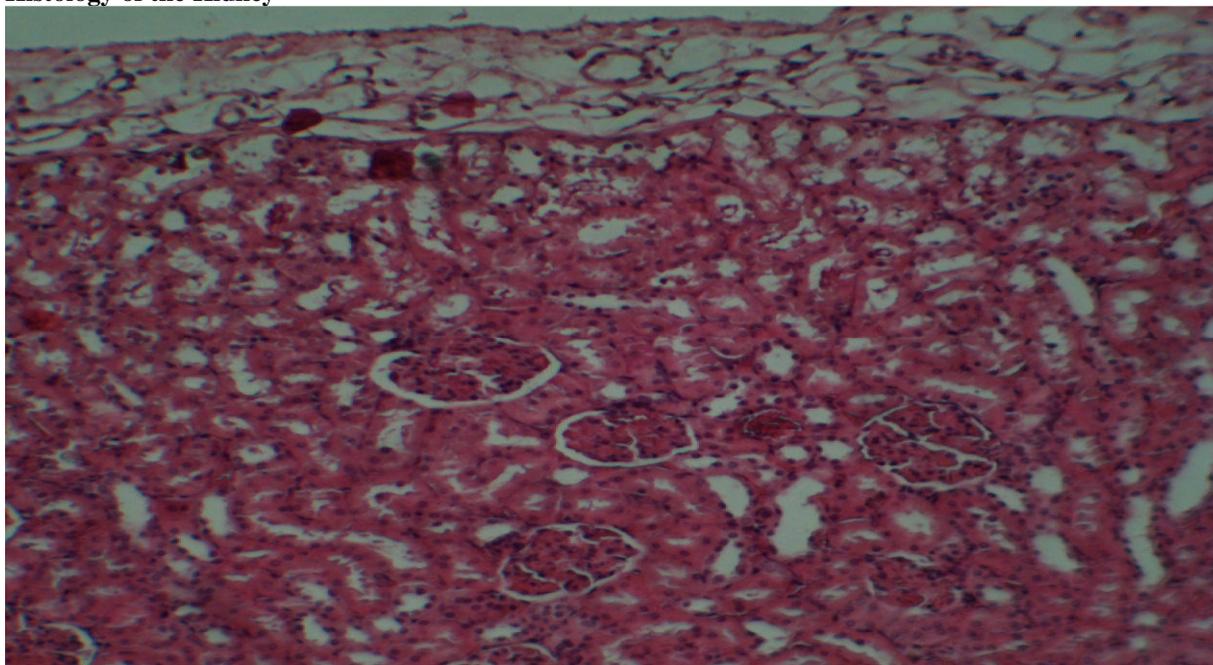


Plate A: The section of the kidney shows normal Glomerulus, Renal Tubulus. Stained H and E mg x 100.

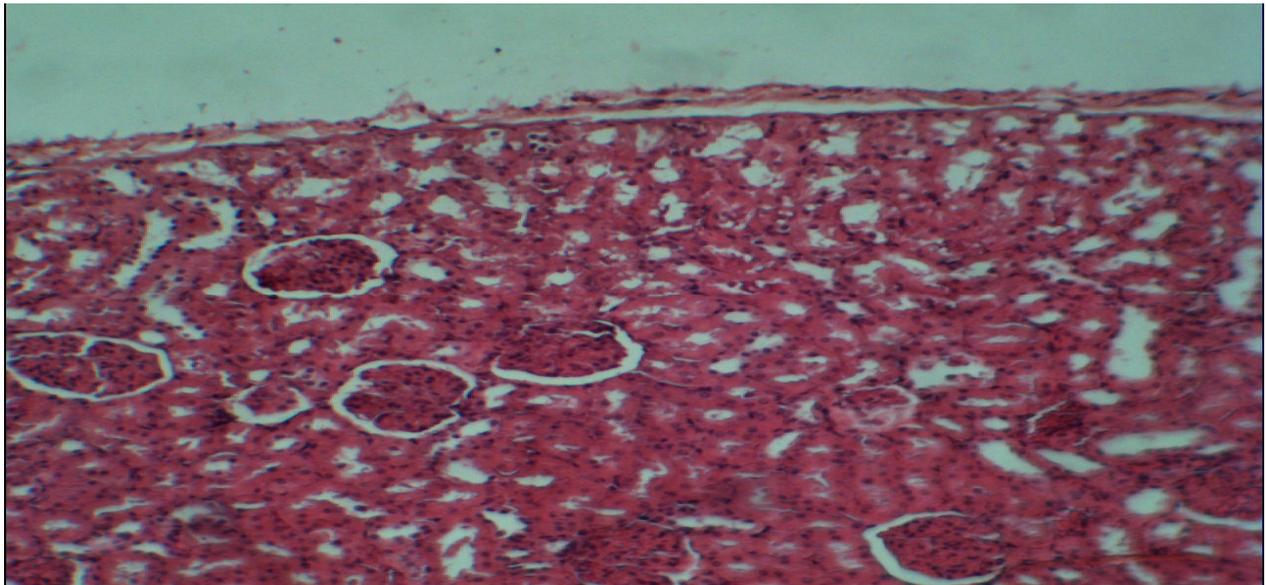


Plate C: The section of the kidney shows the fixtures of Renal Capsules (RC), Glomerulus and the Renal Tubulus. Stained with H and E, mg x 100.

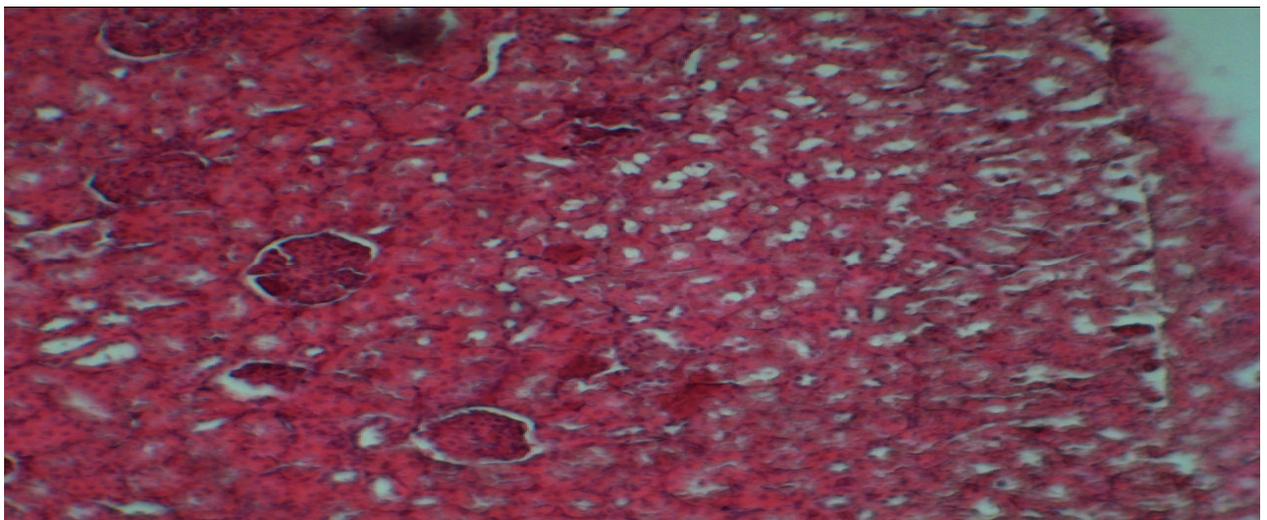


Plate D: The section of the micro-graph shows the histology fixtures of Glomerulus (G) and Renal Tubulus (RT). Stained H and E mg x 100.

HISTOLOGY OF THE LIVER

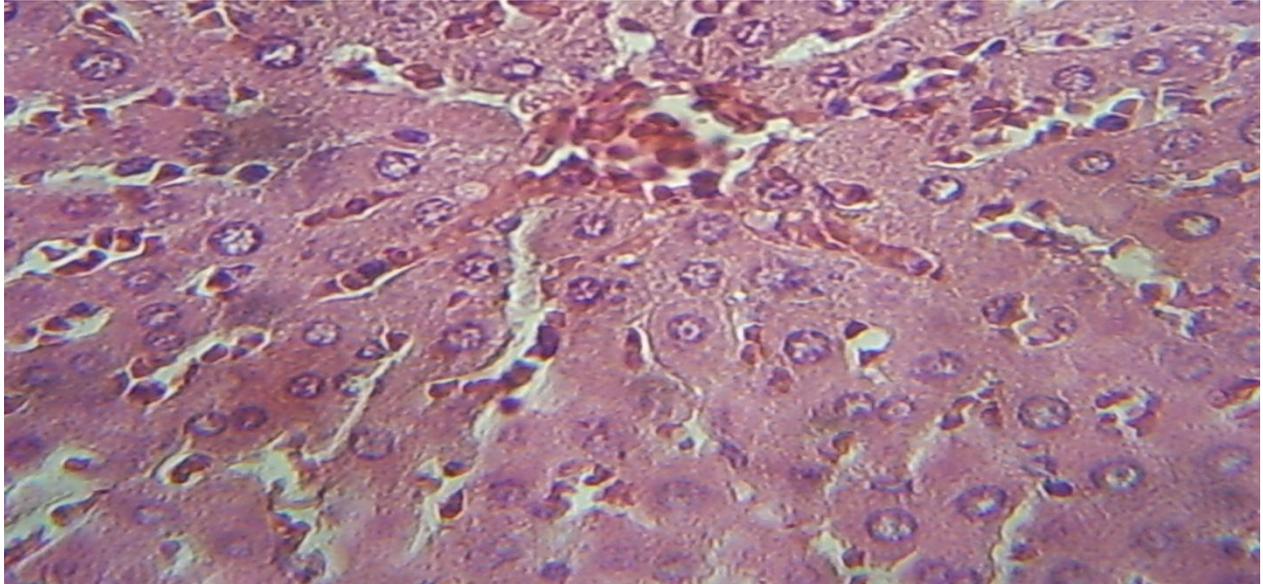


Plate E: The section of the liver shows Central Vein (CV), Nucleus (N), Sinusoids(S), Hepatocytes (H). Stained H and E. mg x 400.

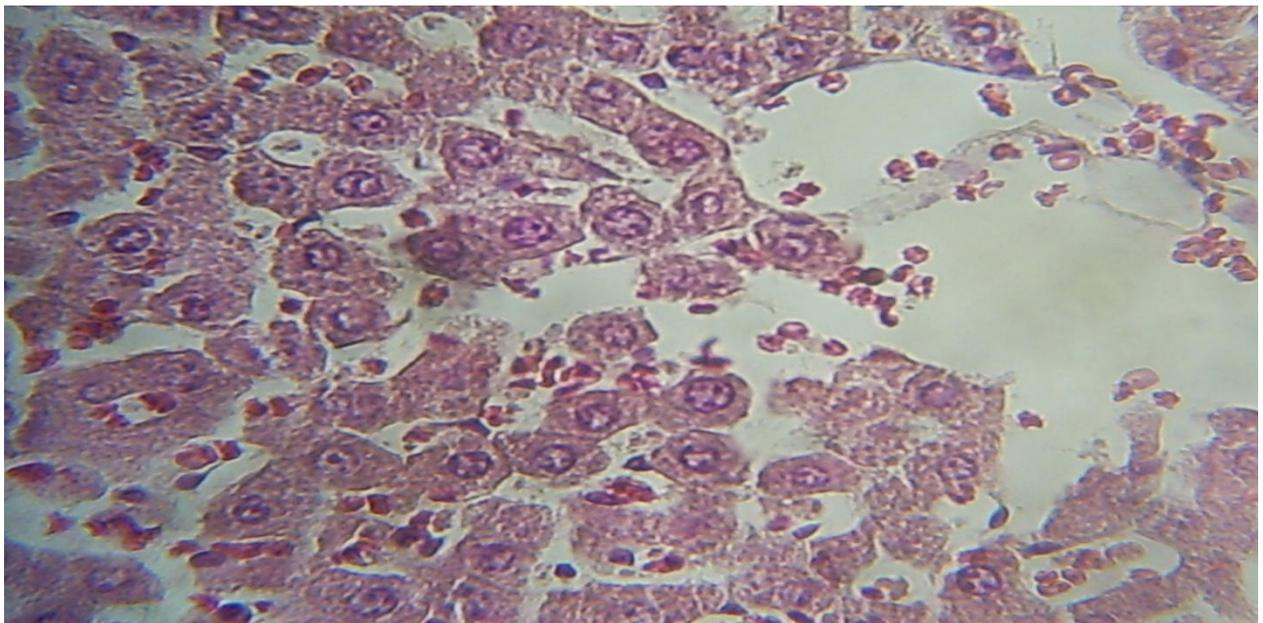


Plate F: The section of the liver shows Hepatocytes, Central Vein and the Sinusoids. Stained H and E mg x 400.

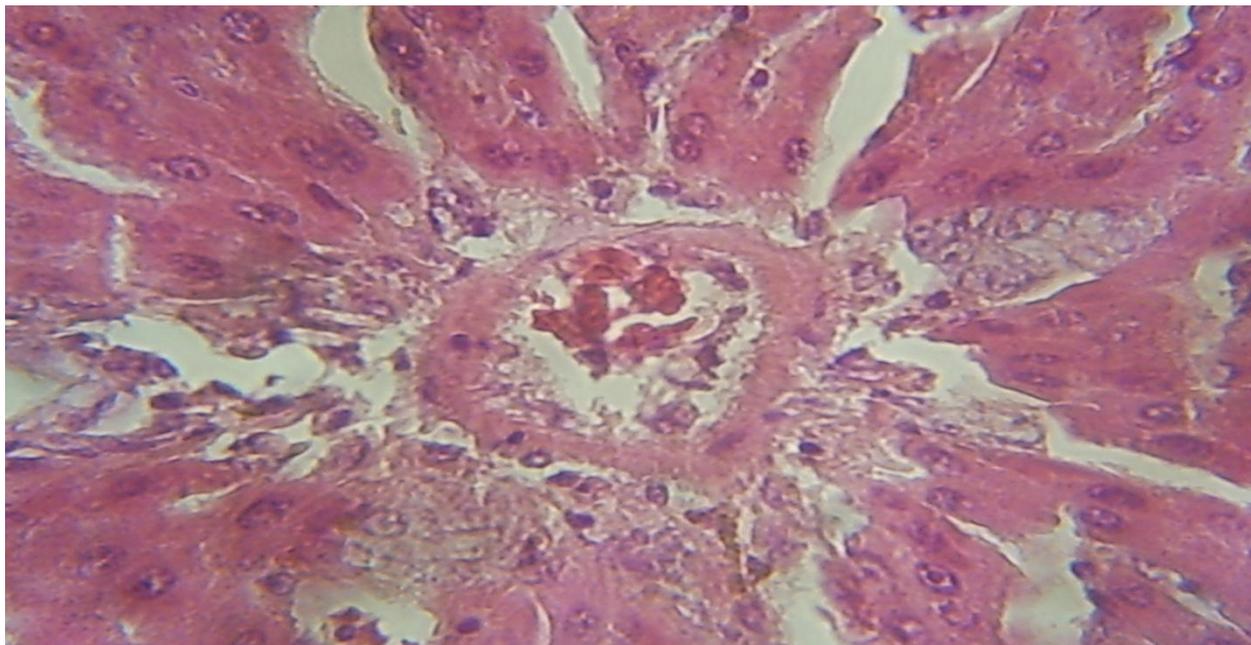


Plate G: The section of the liver shows congestion the Central Vein, Hepatocytes and Sinusoids. Stained H and E mg x 400.

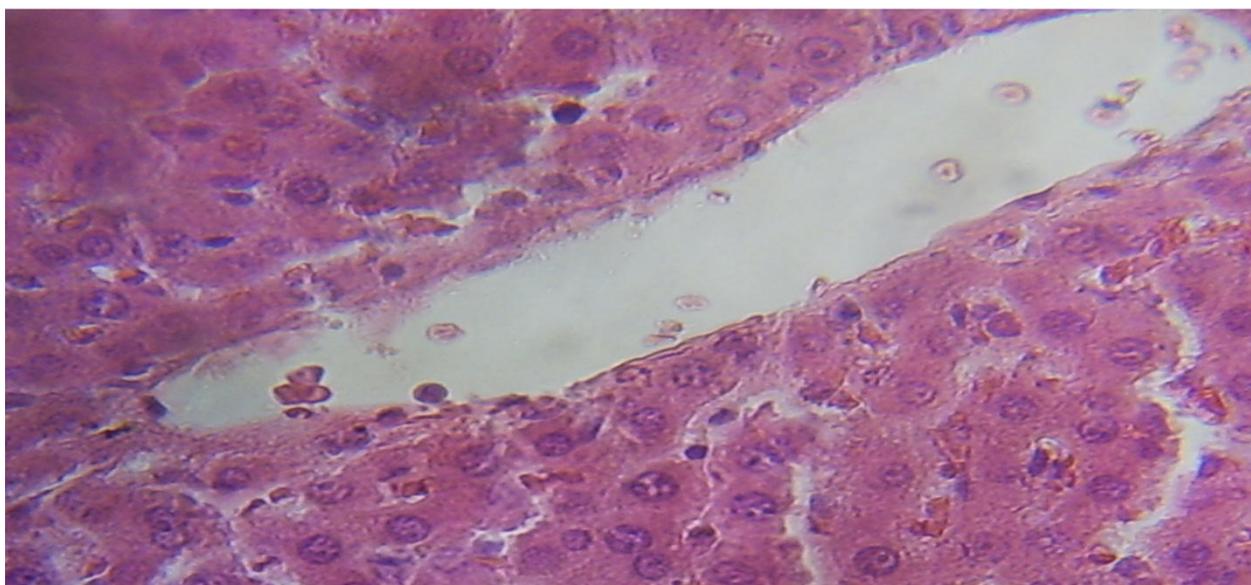


Plate H: The section of the liver shows Hepatocytes, Central Vein, Nucleus and the Sinusoids. Stained H and E mg x 400.

Interpretation of Results

In table 1, statistical evaluations reveal that from first week of administration of ethanolic leaf extract *Moringa oleifera* the animals to the four week before sacrificing the animal, there was a significant change in the weight of the animal when group I (control) was compared with group II, III and IV.

However, in table 2, the HB, white blood cell total and red blood cell count were within the normal range and there was significant ($P < 0.05$) increase in pack cell volume in group II, III and IV when compared with control. Beside similar trends as observed in PCV was also recorded for HB, white blood cell total and red blood cell count.

More so, in table 3, for the biochemical parameters statistical analysis showed that the ethanolic leaf extract of *Moringa oleifera* recorded significant ($P < 0.05$) decrease for Creatinine level in all of the treatment groups (I, II and III) when compared with group one. But there was no significant change in alkaline phosphatase in all the treatment groups (I, II and III) when compared with controls.

The result of the effect of the ethanolic extract of *M. oleifera* leaves on the histology of the liver and

kidney.

In plate A-D in the liver and plate E-H in the kidney, there was no visible lesion or disease observed. But in figure 2 of the dose of 400mg/kg, there is dilation of the central vein and slight discontinuation of hepatocyte which are large polyhedral cells. The sinusoid empties into the central vein line by flat endothelial cells and nucleus. In figure 3 of 600mg/kg shows congestion at the central vein. Sinusoid empties into central vein and the hepatocytes appear normal. In figure 4 with 800mg/kg dose, there was dilation around the nucleus. In the Plate A-D (kidney), the glomerula supported by intact membrane between which are numerous tubules lined by low cuboidal epithelium and blood vessels.

DISCUSSION AND CONCLUSION

The tree, *M. oleifera* (*Moringaceae*), is cultivated widely around the world (Odee, 1998;) and used for various purposes one of which is as a feed supplement to livestock (Fadiyimu *et al.*, 2010). The effect of ethanolic leaves extract of *M. oleifera* on haematological parameters was evaluated, analyzed and interpreted. The assessment of haematological parameters is a biomarker for evaluating the haematotoxic potential of the extract in area of pharmacognosy increase recorded for PCV and HB, WBC_{TOTAL} and RBC count levels for group I, II and III weight following the administration of ethanolic leaf extract of *M. oleifera*. This suggests that the extract contain some bioactive constituents or phytoconstituents which should have imposed or boosted haematopoietic activities. It is also supported by the fact that *M. Oleifera* leaf is rich in terms of nutritional value. Studies have shown that the leaf of *M. oleifera* is an outstanding source of vitamin A, B, C and also among the best plant source of minerals like iron and it is reportedly prescribed for anaemia and lactating mothers in the Philippines and also an excellent source of protein, this is agreement with the findings of, David, *et al.*, (2007), Auwal, *et al.*, 2013, Ewuola, 2012 and Hisham, *et al.*, (2012) who recorded an increase in haematological indices in their research using ethanolic extract of *Moringa oleifera* on rats and rabbits.

Also, the body weights of the rats were significantly increased all through the four week of the experiment. The increase in the body weight of rats might be due to the fact that *M. oleifera* is rich in amino acids, vitamins and minerals particularly iron (Subadra *et al.*, 1997; Faye, 2011). The significant increase in body weights of rats might also be attributed to captivity, where energy expenditure is minimal. This is agreement with the findings of Hisham, 2012 and Ujah, 2012 who also recorded increase in body weight of rats.

The liver is a vital organ in vertebrates and other animals. It is used in the elimination and detoxification of harmful biochemical waste products and toxins. It plays key role in the synthesis of biochemicals that are very vital in body metabolism. The kidney is an important organ of regulation in mammals. They are essential in the urinary and homeostatic function.

The renal and hepatic function of the rats was assessed by serum creatinine and alkaline phosphatase respectively. The results of ethanol leaf extract of *Moringa oleifera* on serum creatinine concentration in mice showed a significant decrease ($p < 0.05$) in serum creatinine concentration in groups (I, II and III). This is agreement with Ugwu (2013) who recorded a decrease in serum Creatinine when *Moringa oleifera* diet was fed on rats. Also, the decrease in creatinine level in group IV (800mg/kg) is in accordance with the finding of (Ajibade, 2012) who recorded a decrease in creatinine level at a dose of 800mg/kg when extract of *Moringa oleifera* seed was administered to rats. The effect of ethanol leaf extract of *Moringa oleifera* on alkaline phosphatase activity in mice showed no significant difference ($p < 0.05$) in alkaline phosphatase in the entire group. Non significant ($p > 0.05$) effect of *Moringa oleifera* and alkaline phosphatase (ALP) is an indication that the treatments have no untoward effect on the rats. This is agreement with the findings of Terzungwe (2013) who observed no effect of *Moringa oleifera* on alkaline phosphatase effect on the health status of the rabbits. Alkaline phosphatase is present in all the tissues throughout the body, but is particularly concentrated in the liver, bile duct, kidney, bone and the placenta. It is therefore not a specific liver marker.

The histological result of ethanolic extract of *Moringa oleifera* at doses (400mg/kg, 600mg/kg and 800mg/kg) on the liver and kidney did not show any visible lesion or disease which implies that administration of *M. oleifera* is non-toxic to animals at low and high doses.

REFERENCES

- Afolayan, AJ, Yakubu MT(2009); Effects of Bulbine natalensis Ball stem extract on the functional indices and histology of the liver and kidney of male Wistar rats .J. Med food; **12**:84-20
- Agaie, B(2004). The role of phytochemicals in optimal health. Journal of the
- Ajibade, T.O, Olayemi, F.O, Arowolo R.O.A (2012). The haematological profile and biochemical effects of ethanol extract of the seeds of *Moringa oleifera* in rats. J. Medicinal Plants Research, Vol 6(4), pp.615-621
- Anwar F, Ashraf M, Bhangar MI. 2005. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. J Am Oil Chem Soc **82**: 45–51.
- Auwal MS, AN Tijjani2, MA Sadiq3, S Saka4, IA Mairiga5, A Shuaibu6, E Adawaren1 & IA Gulani5 (2013)

- Antibacterial and haematological activity of *Moringa oleifera* aqueous seed extract in Wistar albino rats. *Sokoto Journal of Veterinary Sciences*, **11**(1): 28-37.
- David K, Weber Kathleen, Danielson Stan, Wright J and Foley E.(2002). Hematology and serum biochemistry values of rats. *J of Wild life-Dis*, 38(3): 576 – 582.
- Estrella, M.C.P; Mantaring J.B.V; David G.Z (2000). A double blind, randomised controlled trial on the use of malunggay (*Moringa oleifera*) for augmentation of the volume of breastmilk among non-nursing mothers of preterm infants. *Philipp J. Pediatr* **49**: 3–6.
- Ewuola E.O; Jimoh O.A; Atuma O.V; Soipe O.D. (2012).Haematological and Serum Biochemical Response of Growing Rabbits fed Graded Levels of *Moringa Oleifera* Leaf Meal. *World Rabbit Science Association*
- Fadi C, Andrzej P, Ekaterina M and Hayes KC. 2010. Nutrition correlates and Fadiyimu AA, Alokani JA and Fajemisin AN. 2010. Digestibility, nitrogen
- Fahey, J.W(2005). *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1.
- Fahey, J. W; Zalcmann, A.T; Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **56**: 5–51.
- Faye B, Bucheton B, Banuls AL . 2011. Prevalence of leishmania infantum in a rural area of Senegal: analysis of risk factors involved in transmission to humans. *J Trans R. Soc Trop Med Hyg.*, 105: 333 – 340.
- Fuglie, L.J(1999). *The Miracle Tree: Moringa oleifera: Natural Nutrition for the Tropics*. Church World Service, Dakar. 68 pp.; revised in 2001 and published as *The Miracle Tree: The Multiple Attributes of Moringa*, 172 pp.
- Hisham M. Osman1; Mohamed E. Shayoub and Elsiddig M. Babiker (2012).The Effect of *Moringa oleifera* Leaves on Blood Parameters and Body Weights of Albino Rats and Rabbits. *Jordan Journal of Biological Sciences*, *Vo. 5, No 3, Pg 147 – 150*History of Nephrology 2 p. 235 by International Association for the History of Nephrology Congress,
- Jed W; Fahey S. D.(2008). *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic and prophylactic properties medicinal plant extracts. *J Molecules*; **14**: 2167- 2180.
- Mann, A; M. Gbate and A. Ndaumar (2003). *Medicinal and Economic Plants of Nupeland* . Jube-Evans Books and Publications, Bida. P276.
- Maton, Anthea; Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon arner, David LaHart, Jill D. Wright (1993). *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall.
- Morton J.F(1991). The horseradish tree, *Moringa pterigosperma* (Moringaceae). A boon to arid lands. *Econ Bot* **45**: 318–333.
- Nadkarni AK. 1976. *Indian Materia Medica*. Popular Prakashan: Bombay, 810–816.
- Newman, D.J., G.M. Cragg, K.M. Snader, 2000. Newman, D.J., G.M. Cragg, K.M. Snader, 2000. The Influence of Natural Products upon Drug Discovery. *Natural Product Report*, **17**: 215-234.
- Palada M.C, Changl L.C (2003). Suggested cultural practices for *Moringa*. *International Cooperators' Guide AVRDC*.
- Qaiser M(1973). Moringaceae. In *Flora of West Pakistan*, Nasir E, Ali SI (eds). No.38. University of Karachi Press: Karachi, 1–4.
- Ramachandran,C, Peter K.V, Gopalakrishnan, P.K(1980). Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. *Econ Bot* **34**: 276–283.
- Siddhuraju, P; Becker, K (2003). Antioxidant properties of various solvent Pharmacologic properties of *Moringa oleifera*: 2: Screening Copyright © 2006 John Wiley & Sons, Ltd. *Phytother. Res.* **21**, 17–25
- Subadra S, Monica J and Dhabhai D. (1997). Retention and storage stability of beta-carotene in dehydrated *M. oleifera*. *Inter J Food Science and Nutri*, 48:373 – 379.
- The Wealth of India (A Dictionary of Indian Raw Materials and Industrial Products). (1962). *Raw Materials*, Vol. VI: L-M; Council of Scientific and Industrial Research: New Delhi, 425– 429.
- Ugwu Okechukwu, P. C; Nwodo Okwesili, F. C; Joshua Parker E; Odo Christian E; Ossai Emmanuel C and Bawa Aburbakar (2013). Ameliorative Effects of Ethanol Leaf Extract of *Moringa Oleifera* on the Liver and Kidney Markers of Malaria Infected Mice. *Int. J. LifeSc. Bt & Pharm. Vol. 2*,
- Walter F., PhD. Boron (2004). *Medical Physiology: A Cellular and Molecular Approach*. Elsevier/Saunders.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:

<http://www.iiste.org>

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: <http://www.iiste.org/journals/> All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: <http://www.iiste.org/book/>

Academic conference: <http://www.iiste.org/conference/upcoming-conferences-call-for-paper/>

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library, NewJour, Google Scholar

